activity is not impaired, wherein the first DNA fragment has a toluene monooxygenase region of 4.9 kb or less is functionally connected to the promoter to express an active toluene monooxygenase, and the second DNA fragment is functionally connected to the promoter to express a property to enhance the toluene monooxygenase activity.

REMARKS

The claims are 1-48 and 55. The claims have been amended to correct minor informalities and to clarify the intended invention. Reconsideration of the claims is expressly requested.

Applicants affirm the election of Group I, claims 1-48 and 55. Nonelected claims 49-54 have been cancelled.

The Examiner had objected to the specification in that no Sequence Identification numbers were provided in the Figures or in the Brief Description of the drawings. In order to resolve that issue, Applicants have cancelled the applicable portions of the Brief Description and of the Drawings which are deemed redundant to the SEQUENCE LISTING provided in the specification. Applicants have filed herewith

a separate Request for Approval of Drawing Changes in which Figs. 1-14 have been deleted.

The informality in claim 5 has been resolved by correction of indicated syntax error.

A Corrected Computer Readable Form and Amendment of Sequence Listing was entered in the instant filewrapper as Paper No. 9. However, the Examiner has not acknowledged its receipt or entry. Applicants request such a formal acknowledgment in the next communication.

The Examiner has rejected claims 1, 3, 6, 7, 9, 10, 15, 17, 19 and 55 under Rule 112, first paragraph, on the ground that the Examiner was unable to find the description of the invention as now claimed. The Examiner suggests that the specification teaches the structure of only a single representative species, a DNA fragment from *Burkholderia cepacia* KK01. These claims have been amended in accordance with the Examiner's suggestions.

The Examiner states that claims 3, 9, 10, 17 and 55 are equivalent to a claim with no structural limitations wherein an enzyme or protein is defined by the function only and that the specification discloses no identifying characteristics to allow one to recognize a structure as a

member of a gene encoding a toluene monooxygenase activity. These claims have been amended to further define a variant with specific base substitutions. Such substitutions are not deemed to change the property of the protein.

The Examiner states that claims 6, 7 and 19 are equivalent to claiming a gene by its coding regions only and that the claims are insufficiently described. Applicants submit that Example 5 on pages 56-58 provides proper support and specifically describes the sequencing and structure of the toluene monooxygenase gene. The most important is the coding regions to form an active enzyme. It is a well-known technique for a skilled person in the art to design an arrangement of promoter, ribosome-binding region, terminator etc. to express an active enzyme, once the coding region is known. It must be noted that since this monooxygenase gene is derived from a bacterial strain, the role of non-coding region is negligible differing from the eukaryotic genes. The non-transcription/non-translation regions in SEQ ID NO: 1 are not essential. This is clearly shown from specification, Example 6, where tom K - tom P and tom L - tom P are cut out just before the initiation codon and linked directly to trc

promoter and ribosome binding site of an expression vector to express monoxygenase activity in E.coli.

The Examiner has rejected claims 1, 3, 6, 7, 9, 10, 15, 17, 19 and 55 under Rule 112, first paragraph, on the ground that the specification is not enabling for a toluene monooxygenase of an unknown amino acid sequence "homologous" to SEQ ID Nos: 2-7 and a DNA encoding thereof.

Applicants refer the Examiner to Example 5, specifically lines 20-27 on page 57 and lines 1-11 on page 58, which describes the alignment of the segments and the concept that any variation of a segment of the DNA fragment can be made providing the activity of toluene monooxygenase is not impaired. The DNA fragment of Claim 1 is fully specified by the restriction map and by defining that it is derived from B. cepacia KKO1. As clearly stated from specification page 25 line 20 - page 26 line 14, the DNA fragment of the present invention has a restriction map different from that of similar enzymes of other 8 bacterial species. The same is said to claim 15. Claims 6 and 7 are sufficiency definite with the constituting coding regions expressed as SEQ ID Nos.

The Examiner has rejected claims 4, 11, 17 and 55 under Rule 112, second paragraph, as being indefinite because

the relationship between a vector, a promoter and a DNA fragment is unclear. In the present application, a "vector" means a self-replicating (antonomous) DNA molecule which can carry a DNA segment into a host cell and an "expression vector" means a self-replicating DNA molecule plus a promoter for the expression of a DNA segment (see page 26, lines 15 to page 27, line 1, page 59 lines 4-14). Therefore, claims 4, 11, 17 and 55 are correctly directed to a recombinant DNA molecule which comprises a DNA fragment, a vector and a promoter, not "a vector comprising a promoter operably linked to a DNA fragment". The promoter may be defined as being "operably linked to the DNA fragment".

The Examiner has rejected the claimed invention under Rule 101 on the ground that it is directed to non-statutory subject matter. Applicants have amended the claims to recite "an isolated DNA fragment" as suggested by the Examiner.

The Examiner rejects claims 3, 9, 10 and 17 under Rule (102)b as anticipated by Shields et al. First, claim 10 is directed to Tom K, a toluene monooxygenase activating protein, and Shields et al. teaches nothing about Tom K or such a protein. Next, the Examiner alleges that the enzyme

taught in Shields et al. is encoded by a sequence that is about 70% homologous to SEQ ID NO: 1. This homology does not teach the DNA fragment of claims 3, 9 and 17. Let us compare the first three lines of SEQ ID NO: 2 of Shields et al. and that of SEQ ID NO: 3 of the present invention, both being a first unit of monooxygenase. The bold letters are the sequence of the present Invention.

Met Thr Ile Asp Leu Lys Thr Arg Glu Ile Lys Pro Leu Arg His Thr

Met Thr Ile Glue Leu Lys Thr Val Asp Ile Lys Pro Leu Arg His Thr

Tyr Thr His Val Ala Gln Tyr Ile Gly Ala Asp Lys Ala Ala Ser Arg

Phe Ala His Val Ala Gln Asn Ile Gly Gly Asp Lys Thr Ala Thr Arg

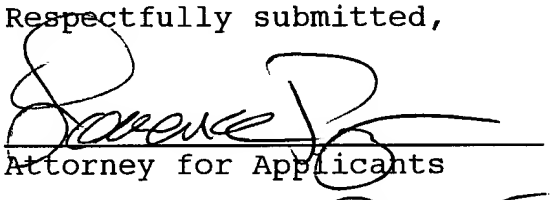
Among 16x3 pairs, different pairs are arg-val, glu-asp, tyr-phe, thr-ala, tyr-asn, ala-gly, ala-thr, ser-thr, thr-met, val-met, ala-gln, and ala-glu. In the amino acid sequence is varied to have the same amino acid according to the rule permitted by the claims, e.g., aromatic to aromatic, basic to basic, acidic to acidic, aliphatic to aliphatic, sulfur-containing to sulfur-containing, hydroxy to hydroxy, there will still remain 7 pairs: arg-val, thr-ala, tyr-asn, ala-thr, thr-met, val-met, and ala-glu. Thus, the variant

DNA fragments of claims 3, 9 and 17 are not anticipated by the sequence taught in Shields et al.

In view of the above amendments and remarks, Applicants submit that all of the Examiner's concerns are now overcome and the claims are now in allowable condition. Accordingly, reconsideration and allowance of this application is earnestly solicited.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should continue to be directed to our below listed address.

Respectfully submitted,



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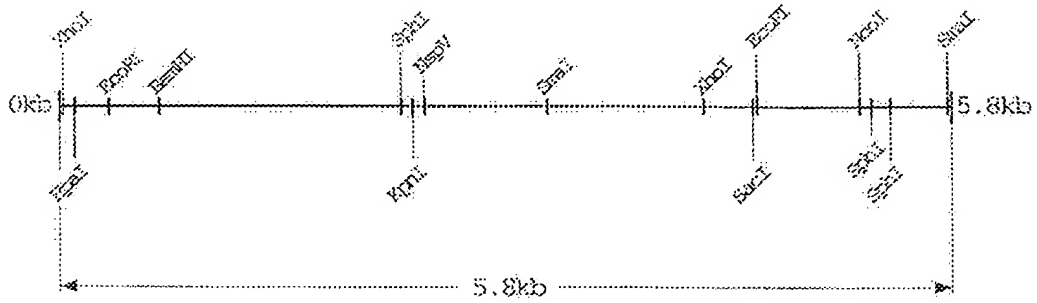
VERSION WITH MARKINGS TO SHOW CHANGES
MADE TO CLAIMS 1-3, 5-11, 17 AND 55

1. (Amended) [A] An isolated DNA fragment of about 5.8 Kb containing a toluene monooxygenase gene, having 1 BamHI restriction site, 2 EcoRI restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site, no PvuII restriction site, no ScaI restriction site, no Sse8387I restriction site, no StuI restriction site, and no XbaI restriction site, and having a restriction map of:



, said isolated DNA-fragment derived from Borkholderia cepacia KK01.

2. (Amended) The isolated DNA fragment [according to claim 1], said isolated DNA-fragment derived from Borkholderia cepacia KK01 wherein the DNA fragment has a nucleotide sequence of SEQ ID NO: 1 in the Sequence Listing.

3. (Amended) [A] An isolated DNA fragment having a nucleotide sequence of SEQ ID NO: 1 with [deletion,] substitution, [and/or addition of one or more nucleotides encoding a protein having a toluene monooxygenase activity] with substitution of at least one nucleotide, said substitution resulting in 1) no amino acid change with code degeneration, or 2) amino acid substitution only between aliphatic amino acids, between sulfur-containing amino acids, between hydroxy amino acids, between aromatic amino acids, between basic amino acids, and between acidic amino acids.

5. (Amended) The recombinant DNA fragment according to claim 4, wherein the vector can be maintained or [replicate] replicated in a bacterium.

6. (Amended) [A] An isolated DNA fragment containing a region encoding a toluene monooxygenase, the region comprising a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO: 3, a second sequence encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7 of the Sequence Listing, and the first to fifth sequences are aligned so that expressed TomL - TomP polypeptides can form an active monooxygenase protein.

7. (Amended) [The] An isolated DNA fragment according to claim 6, wherein no spacer sequence is present between the first to fifth sequences or at least one spacer sequence is present between the first to fifth sequences.

8. (Amended) [The] An isolated DNA fragment according to claim 6 or 7, further comprising a sequence encoding a polypeptide TomQ having an amino acid sequence of SEQ ID NO: 8 in the Sequence Listing.

9. (Amended) [A] An isolated DNA fragment containing a region encoding a toluene monooxygenase, wherein the region comprises a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID N0: 3, a second sequence encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7, and the first to fifth sequences are aligned so that expressed TomL - TomP polypeptides can form an active monooxygenase protein;

wherein in at least one of the first to fifth sequences of the DNA fragment[, deletion,] substitution[, and/or addition of one or more nucleotides are present in the proviso that the toluene monooxygenase protein is active] with substitution of at least one nucleotide, said substitution resulting in 1) no amino acid change with code degeneration, or 2) amino acid substitution only between aliphatic amino acids, between sulfur-containing amino acids, between hydroxy amino acids, between aromatic amino acids, between basic amino acids, and between acidic amino acids.

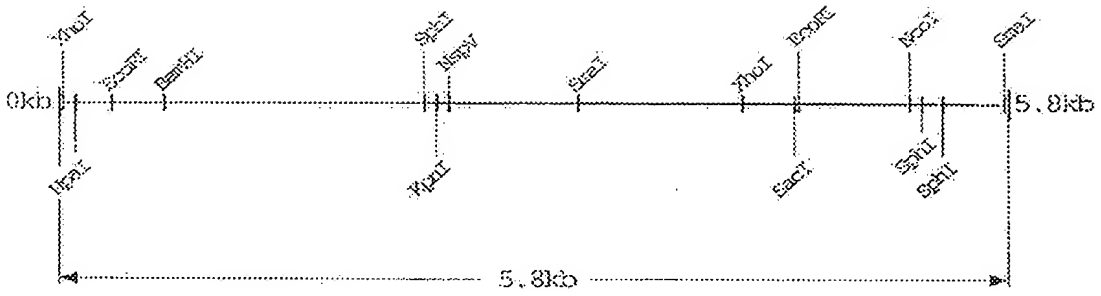
10. (Amended) [A] An isolated DNA fragment comprising a region encoding a polypeptide TomK, the polypeptide TomK having an amino acid sequence of SEQ ID NO: 2, [and a property to enhance the toluene monooxygenase activity of a protein comprised of at least TomL to TomP; or a region encoding a variant TomK in which the amino acid sequence of SEQ ID NO: 2 is altered with the proviso that the property to enhance the toluene monooxygenase activity is not impaired] with substitution of at least one nucleotide, said substitution resulting in 1) no amino acid change with code degeneration, or 2) amino acid substitution only between aliphatic amino acids, between sulfur-containing amino acids, between hydroxy amino acids, between aromatic amino acids, between basic amino acids, and between acidic amino acids.

11. (Twice Amended) A recombinant DNA comprising a vector, a promoter, and the DNA fragment according to any one of claims 6, 7 or 9, [and] wherein the vector and the promoter are functionally ligated to the DNA fragment to enable expression of the toluene monooxygenase encoded by the DNA fragment.

15. (Amended) A transformant obtained by introducing a recombinant DNA into a host microorganism, the recombinant DNA

comprising a vector enabling maintenance or replication in a host and a DNA fragment of about 5.8 Kb containing a toluene monooxygenase gene, having 1 BamHI restriction site, 2 EcoRI restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site, no PvuII restriction site, no ScaI restriction site, no Sse8387I restriction site, no StuI restriction site, and no XbaI restriction site, and having a restriction map of DNA fragment of about 5.8 Kb containing a toluene monooxygenase gene having 1 BamHI restriction site, 2 EcoRI restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site, no PvuII restriction site, no ScaI restriction site, no Sse8387I restriction site, no StuI restriction site, and no XbaI restriction site, and having a

restriction map of:



, said DNA-fragment derived from Borkholderia cepacia KK01.

17. (Amended) A transformant obtained by introducing a recombinant DNA into a host microorganism, where the recombinant DNA comprises a vector enabling maintenance or replication in a host, and a DNA fragment ligated thereto having a nucleotide sequence of SEQ ID NO: 1 of the Sequence Listing with deletion, substitution and/or addition of one or more nucleotides, still encoding an active toluene monooxygenase, wherein the DNA fragment has a toluene monooxygenase region of 4.9 kb or less.

55. (Amended) A recombinant DNA comprising a vector, a promoter, a first DNA fragment being the DNA fragment of any one of claims 6, 7 or 9, and a second DNA fragment, said second DNA fragment comprising a region encoding a polypeptide TomK having an amino acid sequence of SEQ ID NO: 2, and a property to

enhance the toluene monooxygenase activity of a protein comprised of at least TomL to TomP; or a region encoding a variety of TomK in which the amino acid sequence of SEG ID NO. 2 is altered with the proviso that the property to enhance the toluene monooxygenase activity is not impaired, wherein the first DNA fragment has a toluene monooxygenase region of 4.9 kb or less is functionally connected to the promoter to express an active toluene monooxygenase, and the second DNA fragment is functionally connected to the promoter to express a property to enhance the toluene monooxygenase activity.